Journal of Evidence-Based Complementary & Alternative Medicine
2016, Vol. 21(4) NP85-NP90

DOI: 10.1177/2156587216642101
cam.sagepub.com

Effect of Hydroalcoholic Extract of *Rosmarinus officinalis* L. Leaf on Anxiety in Mice

Mosayeb Noori Ahmad Abadi, MD[1], Mohsen Mortazavi, MD[1], Navid Kalani, BSc[2], Hadi Zare Marzouni, MSc[3], Wesam Kooti, MSc[4], and Sara Ali-Akbari, MSc[4]

Abstract

In today's stressful world, psychopathy (especially anxiety) is receiving increased importance. Most of the drugs used to treat this disease have several side effects. Medicinal plants derived from natural products have fewer side effects and can be used in the treatment of this disease. The aim of this study was to evaluate the effect of the hydroalcoholic extract of *Rosmarinus officinalis* L. on anxiety in mice. In this experimental study, 50 male mice were randomly divided into 5 groups. To evaluate anxiety, the Elevated Plus Maze test was performed. The control group received normal saline, the positive control group received diazepam (1 mg/kg) as intraperitoneal injection, and the experimental groups received doses of 100, 200, and 400 mg/kg body weight of rosemary extract. The data were analyzed using SPSS 15 and ANOVA statistical tests. The results show that rosemary extract dose-dependently increases the mice spending time and the entries number of mice in plus maze open arms (indicating less stress). This effect at a dose of 400 mg/kg was similar to diazepam, which, in comparison to the control group, was statistically significant ($P < .01$), while the evaluation of locomotor activity in treated groups, compared with the control groups, showed no significant difference ($P > .05$). On the other hand, the rosemary extract, similar to the standard drug diazepam, showed an anti-anxiety effect. This effect is probably due to the presence of flavonoids in this plant and their antioxidant property.

Keywords

anxiolytic, *Rosmarinus officinalis*, mice

Received November 10, 2015. Received revised February 24, 2016. Accepted for publication March 5, 2016.

In today's stressful world, psychopathy (especially anxiety) has received increased importance because of its high prevalence.[1] Anxiety is a feeling of fear and discomfort caused by no specific reason, which undermines peoples' function and is the main and most common symptom of psychopathy. During the history of psychology, this disorder has always been considered due to adverse consequences; therefore, in this field many studies have been carried out and huge financial resources and human resources have been allocated to treat anxiety.[2] According to World Health Organization, estimation of the prevalence of this disorder is increasing in developing countries. The World Health Organization estimates anxiety as one of the top mental disorders, with a population of 500 million suffering worldwide.[3] According to recent studies of the Anxiety and Depression Association of America, of every 10 US adults 7 claim that they have experienced either anxiety or stress at least on a daily basis and at an intermediate level.[4] According to surveys in Iran, anxiety, with a prevalence of 2.3%, was at the top of mental disorders, and on this basis 20.8% of those surveyed suffer from symptoms of anxiety.[3] Due to the different side effects and harmful effects of some industrial medicines, many patients prefer alternative and complementary medicines for treatment. A study in a population more than 2000 people demonstrated that more than half of those who suffer from anxiety attacks use medicinal herbs or other complementary therapies to treat their disorders. Although all herbal medicines are not safe, most herbal products that are used in

[1] School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2] Medical Ethics Research Center, Jahrom University of Medical Sciences, Jahrom, Iran
[3] Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran

Corresponding Author:
Wesam Kooti, Department of Immunology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.
Email: wesamkooti@gmail.com

psychotherapy are relatively safe and have fewer side effects than chemical drugs, such as antidepressants.[5]

Among the effective medicinal plants used in the treatment of mental disorders, rosemary can be noted. Rosemary (*Rosmarinus officinalis* L.) belongs to the Lamiaceae or Labiatae family and is a perennial plant with a shrub form and evergreen branches that rise to a height of 2 meters by mast and are occasionally lying on the ground as branches; they are green and have a fragrance. The leaves of this plant and the main branches are collected in the spring and summer for pharmaceutical purposes. This plant is native to Mediterranean and South European countries, and is also cultivated in Iran.[6]

Volatile oil is the main component derived from the leaves of this plant, and its most important compounds include tricicline, 1,8-cineole, camphor, camphene, and bornyl acetate, which vary depending on the geographical conditions and the altitude of cultivation. The hydromethanolic plant extract contains flavonoids such as luteolin, phenolic acid, diterpenes, triterpenes, tannins, and resins.[7]

Clinical studies indicate that rosemary has multiple pharmacological properties and helps in reducing high blood glucose,[8] increases secretion of bile,[9] and has antimicrobial, anti-inflammatory, antifungal,[10] smooth muscle relaxant, antioxidant, anti–skin allergy,[11] and antimutagenic properties.[12] The plant is valuable in the pharmaceutical and medical industry due to its antimicrobial, antimutagenic, and chemical prevention agent properties. Rosemary's essential oil is used for flavoring food, is used in the cosmetic industry, and is also used as disinfectants and pesticides.[6] This plant's essence increases blood circulation in organs, has antirheumatic effects, and reduces neuralgia.[13] Rosemary's analgesic effects are due to opioid receptors. In ancient medicine, the plant has been used for treating anxiety.[11,14]

Most of the drugs used to treat anxiety cause several adverse reactions such as anticholinergic effects, arrhythmias, and hypotension.[15] The selective serotonin reuptake inhibitor antidepressant family is a new drug class used in therapy and is used as a first-line treatment, for example, citalopram at 20 to 60 mg per day doses, and but has side effects such as nausea, vomiting, sexual dysfunction, agitation, and insomnia.[16] On the other hand, benzodiazepines as an adjunct drug is used in the treatment of anxiety and insomnia in depressed patients. This drug should be used with caution because of the risks of drug dependency.[17] Although many drugs are used to treat mental illness, physicians believe that these drugs' therapeutic responses are not enough, and patients are unable to tolerate their side effects; however, herbal medicines have fewer side effects and can be used alone or as a supplement in the treatment of this disorder.[18] The effectiveness of the different medicinal plants in treating depression has been demonstrated. Studies have shown that plants of the Lamiaceae family are effective in treating mental disorders and anxiety.[19] With regard to the effects of rosemary in the treatment of mental disorders using traditional medicine, we decided to investigate rosemary's potential effects on anxiety.

Materials and Methods

Animals

To perform this experimental study, 50 mice (weight range of 25-35 g) with an average age of 8 weeks were obtained from the Pasteur Institute (Tehran, Iran). The animals did not have any infectious disease and all were healthy. The animals were kept in plastic cages in a room at a temperature of about 22°C to 24°C, suitable environmental conditions, and 12 hours of darkness/light; also, they were given food and water indefinitely. During the study, all the other mice were kept in the same conditions and were unchanged. The study followed ethics and animal care manual protocols published by the National Institutes of Health.

Extraction

The rosemary plant was obtained from an area surrounding Sanandaj and was then identified and confirmed by a member of Shahrekord Plant Research Center as *Rosmarinus officinalis* L species. The plant sheets were washed in flowing water and then were dried for 2 days at room temperature. In addition, the Maceration method was used for extraction. For this purpose, the leaves and twigs of the rosemary plant after separation from other parts were ground in an electric mill. Then 500 gr of the powder in combination with 70% alcohol (2 L) was soaked for 72 hours in an environment protected from light. After filtering the resulting liquid by a rotary device (distillation in a vacuum) twice, it was incubated at 40°C to evaporate the solvent and then the extract was dried after distillation.[20] All stages of extraction were done in sterilized containers. Due to the required concentration the extract was diluted with normal saline (Daru Pakhsh Company, Tehran, Iran) and doses of 100, 200, and 400 mg/kg body weidht were prepared.

Experiments

The positive control group was administered diazepam at a dose of 1 mg/kg. Also, for the experimental groups, doses of 100, 200, and 400 mg/kg body weight of rosemary hydroalcoholic extract was administered, while the control group received normal saline. All animals received 0.2 mL of drug intraperitoneally. This test was performed according to the method introduced by Walf and Frye.[21] The Elevated Plus Maze device (Stoelting Co, Wood Dale, IL) is an unconditional model used to produce and measure anxiety, determine the drug's anxiety and antianxiety effects, and does not require any practical training and learning. This device is currently the most reliable means of production and evaluation of anxiety in animal models. The device consists of 2 open and 2 closed arms against each other to form a "+" (40 cm long, 10 cm wide). The 2 arms have no walls (open arms), but the closed arms are surrounded by 40 cm walls. At the center is a 10 × 10 cm box. The device is placed 50 cm above the ground, so that a 40 Watt bulb to a height of 1 meter is mounted on top of it. After 45 minutes of injection, each rat in the middle of the device is placed on its head into the open arms, and testing is done for 5 minutes. When the animals enter the open arms by seeing height, they get scared and quickly go to the closed arm to be protected. The best criterion to measure anxiety is by the presence and number of open arm entries. It should be noted that the presence span and the number of entries into the open arm has an inverse relationship to the anxiety level. That is, the more the duration of presence or the entries in the arm the less the anxiety. The arrival of every 4 feet of the animal was considered as

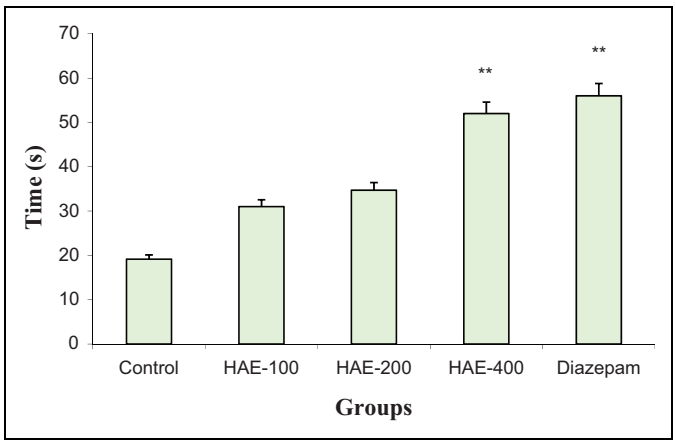

Figure 1. Comparison of different doses of hydroalcoholic extracts of rosemary and diazepam to control group on spent time in the open arm of the Elevated Plus Maze in mice.

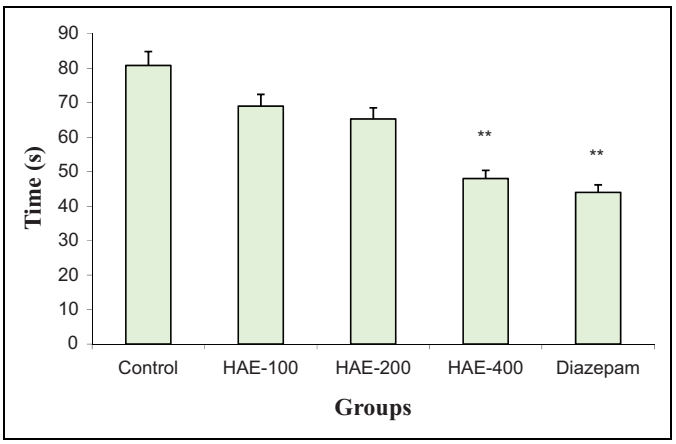

Figure 2. Comparison of different doses of hydroalcoholic extracts of rosemary and diazepam to control group on spent time in the closed arm of the Elevated Plus Maze in mice.

inclusion and exclusion criteria to the different parts of the system.[22] Within 5 minutes, the time spent in the open and closed arms and the number of entries to both arms were recorded separately, and the time spent in open arms (%OAT; %Open Arm Time), the percentage of entries into open arms (%OAE; %Open Arm Entries), and the Locomotor Activity were calculated as standard factors of the anxiety measure.

%Open Arm Time (%OAT): Time spent in open arms divided by the time spent in both arms $\times$ 100

%Open Arm Entries (%OAE): Number of entries into the open arms divided by the number of entries in both arms $\times$ 100

Locomotor Activity : Total entry into the open and closed arms

Each animal was tested only once in order to not let the animal learn. The device numbers of the entries in both the opened and the closed arm are locomotor activities. Whenever there are more entries into the arms, they are signs for increased Locomotor Activity. This test is designed to differentiate between the effect of antianxiety drugs and the effect of activity reactions.[23] Following each test, all parts of the system that had been in contact with animals were cleaned with cotton and alcohol and then dried.

Statistical Analysis

Data were analyzed by using SPSS15 software, one-way ANOVA was followed by least significant difference tests for post hoc analysis. Differences with $P < .05$ were considered significant. Data were expressed as mean $\pm$ SEM.

Results

Comparison between the means and standard deviations of measured variables in different groups is presented in Figures 1 to 5. The results showed that the hydroalcoholic

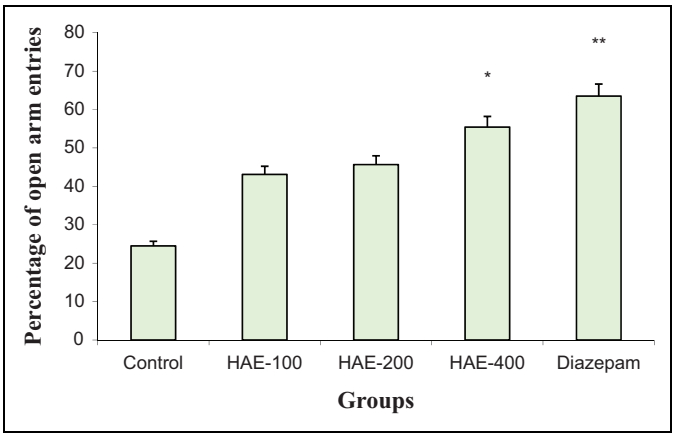

Figure 3. Comparison of rosemary hydroalcoholic extract and diazepam at different doses to control group on percentage of entries into the Elevated Plus Maze open arms in mice.

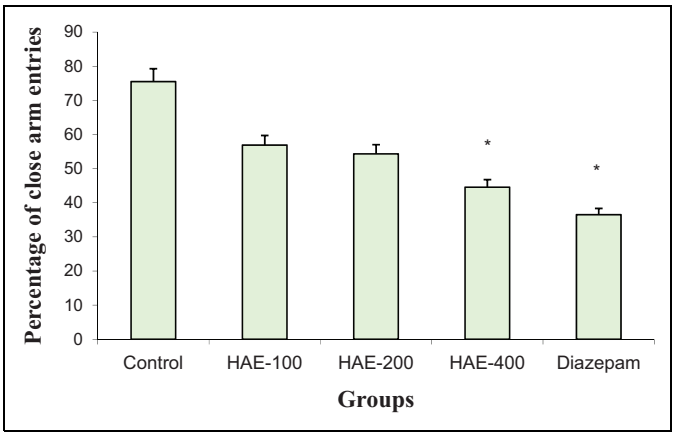

Figure 4. Comparison of rosemary hydroalcoholic extract and diazepam at different doses to control group on percentage of entries into the Elevated Plus Maze closed arms in mice.

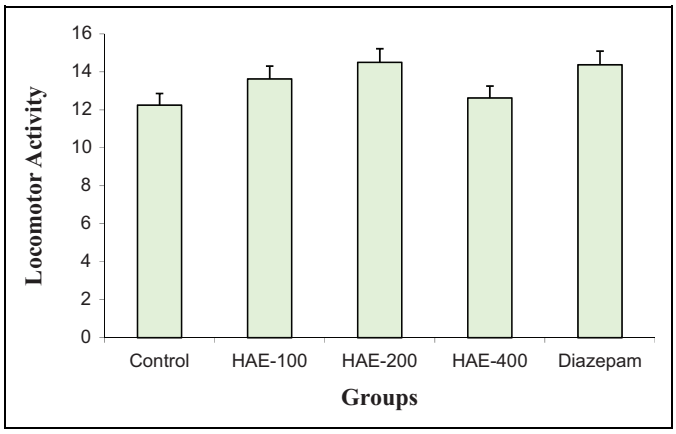

Figure 5. Comparison of rosemary hydroalcoholic extract and diazepam at different doses to control group on the Elevated Plus Maze locomotor activity in mice.

extract of rosemary increases the time mice spent in plus maze open arms (indicating less stress). This effect was dose-dependent, and at higher doses the results were more effective. The effect of the extract on survival time of the mice in the open arms at a dose of 400 mg/kg was similar to the effect of diazepam at a dose of 1 mg/kg, which in comparison to the control group was significant ($P < .01$; Figure 1). Furthermore, under treatment, the time mice spent in the closed arm dose-dependently decreased, and at a dose of 400 mg/kg was similar to the effect of diazepam ($P < .01$; Figure 2). In addition, in this test, the spent time in the open arms and the number of entries was calculated so that the results were in line with OAT, and the treated groups in comparison to the control group entered more number of times into the open arm ($P < .05$; Figure 3). Also, the number of entries of experimental groups mice into the closed arm decreased dose dependently ($P < .05$; Figure 4).

Locomotor activity in the treated groups was compared with the control group, and the positive control group showed no significant difference ($P > .05$; Figure 5).

Discussion

The aim of this study was to evaluate the effect of the hydroalcoholic extract of rosemary on anxiety in mice. The results showed that rosemary in a dose-dependent manner increases the entry and the time spent by the mice in the Plus Maze open arm (indicating less stress). In addition, it does not have a significant effect on locomotor activity. The effect of this herb in high doses was similar to that of diazepam. The rosemary plant contains luteolin, phenolic acid, diterpenes, triterpenes, tannins, and resins. These compounds may increase the plant's effects on different areas of the central nervous system. Diazepam, as a benzodiazepine drug, has proven sedative effects on the central nervous system; also, it is considered as an anxiolytic drug. Diazepam through interaction with brain GABA receptors, particularly in the reticular formation of the midbrain, can cause sedative and anxiolytic effects.[24] Studies have shown a wide role of neurochemical systems in the phenomenon of anxiety. But the GABAergic system and the GABA A receptor are the most involved systems in anxiety. After ligand-receptor binding, the chlore channel opens, and it causes inhibition and hyperpolarization of neurons.

Rosemary is rich in flavonoids; flavonoids serve as ligand for central nervous system receptors and, thus, might act like benzodiazepines. This hypothesis was confirmed by behavioral studies that were done on anxiety behavior and sedation and seizures in animal models.[25] Flavonoids, especially apigenin (one of the active constituents of rosemary), are able to cross the blood-brain barrier, and as a positive and allosteric regulator it enhances GABA effects on GABA receptors; unlike diazepines, they do not cause dependence after an anti-anxiety effect.[26]

Luteolin is another flavonoid in rosemary that also has sedation and anti-anxiety effects by binding to GABA receptors. Another effective combination is carnosic acid, and its antidepressant effect was proved by Kumar et al.[27] Rosemary's sedative effects are possibly due to its antioxidant property.[28] Free radicals such as superoxide, hydrogen peroxide, hydroxyl, and lipid peroxidation radicals cause damage to nerve tissues, and as a result causes neurodegenerative diseases such as epilepsy, schizophrenia, Parkinson's disease, Alzheimer's disease, and exacerbate mental illness, anxiety, and stress.[29]

Parisa et al showed that rosemary has powerful antioxidant effects.[30] Probably this plant with its antioxidant properties protects the brain and other tissues from damage from free radicals. Probably rosemary through oxidative stress reduction and cell protection against apoptosis causes serotonergic neuron protection and anxiety reduction. Rosemary with its anti-inflammatory properties prevents inflammation-causing agents such as tumor necrosis factors-α, denaturation of proteins, and decreases dopaminergic and serotonergic neuron damage.

Dopamine is able to stimulate ACTH secretion by the pituitary, and this neurotransmitter plays a role in reducing anxiety. Therefore, rosemary through MAO might probably create protective effects on neurons and cause anxiety reduction. In the study by Hosseinzadeh et al, it was observed that the hydroalcoholic extract of rosemary is similar to diazepam, and it reduces symptoms of morphine deprivation syndrome in mice.[31] In that study, it was found that some fractions of the plant reduces emotional states and anxiety, and also by reducing the action of GABA receptors the leaning is reduced.

Conclusions

The present study showed that the hydroalcoholic extract of rosemary, especially in high doses, has anxiolytic effects. This effect is probably due to the presence of flavonoids and their antioxidant properties in removing free radicals. Also, the presence of compounds such as apigenin, carnosic acid, and luteolin are involved in the anti-anxiety effects of rosemary. It is recommended that subsequent studies look more closely at the rosemary plant's mechanism of action, and also its active

ingredients, in the hope that in the future the efficacy of this plant could be used in clinical studies.

Acknowledgments

This work was supported by the Medical Plant Research Center of Shahrekord University of Medical Sciences.

Author Contributions

Study concept, design, and critical revision of the manuscript for important intellectual content: Wesam Kooti, Sara Ali-Akbari, Mosayeb Noori Ahmad Abadi, and Mohsen Mortazavi. Drafting of the manuscript and advisor: Wesam Kooti. Conducting the experiments: Navid Kalani and Hadi Zare Marzouni.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The results provided are from a research plan approved with the number 1237 and was funded by the Deputy of Research and Technology of Shahrekord University of Medical Sciences.

Ethical Approval

The authors declare that the experiments done on animals were conducted in accordance with local ethical committee laws and regulations with regard to care and use of laboratory animals.

References

1. Maurer J, Rebbapragada V, Borson S, et al. Anxiety and depression in COPD: current understanding, unanswered questions, and research needs. *Chest*. 2008;134(4 suppl):43S-56S.
2. Erickson KI, Miller DL, Roecklein KA. The aging hippocampus: interactions between exercise, depression, and BDNF. *Neuroscientist*. 2012;18:82-97.
3. Andrews G, Cuijpers P, Craske MG, McEvoy P, Titov N. Computer therapy for the anxiety and depressive disorders is effective, acceptable and practical health care: a meta-analysis. *PLoS One*. 2010;5:e13196.
4. Aluoja A, Leinsalu M, Shlik J, Vasar V, Luuk K. Symptoms of depression in the Estonian population: prevalence, sociodemographic correlates and social adjustment. *J Affect Disord*. 2004; 78:27-35.
5. Wang J, Wan YG, Sun W, Zhang HL, Chen P, Yao J. Progress in Japanese herbal medicine in treatment of chronic kidney disease [in Chinese]. *Zhongguo Zhong Yao Za Zhi*. 2008;33: 1348-1352.
6. Zargari A. *Medicinal Plants*. 6th ed. Vol. 3. Tehran, Iran: Tehran University Publication.
7. Brewer MS. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Compr Rev Food Sci Food Saf*. 2011;10:221-247.
8. al-Hader AA, Hasan ZA, Aqel MB. Hyperglycemic and insulin release inhibitory effects of Rosmarinus officinalis. *J Ethnopharmacol*. 1994;43:217-221.
9. Cho HJ, Yoon IS. Pharmacokinetic interactions of herbs with cytochrome P450 and P-glycoprotein. *Evid Based Complement Altern Med*. 2015;2015:736431.
10. Mugnaini L, Nardoni S, Pinto L, et al. In vitro and in vivo antifungal activity of some essential oils against feline isolates of *Microsporum canis*. *J Mycol Med*. 2012;22:179-184.
11. Tabassum N, Hamdani M. Plants used to treat skin diseases. *Pharmacogn Rev*. 2014;8(15):52-60.
12. Moreno S, Scheyer T, Romano CS, Vojnov AA. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radic Res*. 2006;40: 223-231.
13. Luqman S, Dwivedi GR, Darokar MP, Kalra A, Khanuja SP. Potential of rosemary oil to be used in drug-resistant infections. *Altern Ther Health Med*. 2007;13(5):54-59.
14. Machado DG, Cunha MP, Neis VB, et al. *Rosmarinus officinalis* L. hydroalcoholic extract, similar to fluoxetine, reverses depressive-like behavior without altering learning deficit in olfactory bulbectomized mice. *J Ethnopharmacol*. 2012;143: 158-169.
15. Schulz P, Macher JP. The clinical pharmacology of depressive states. *Dialogues Clin Neurosci*. 2002;4:47-56.
16. Koenig AM, Thase ME. First-line pharmacotherapies for depression—what is the best choice? *Pol Arch Med Wewn*. 2009;119: 478-486.
17. Mann JJ. The medical management of depression. *N Engl J Med*. 2005;353:1819-1834.
18. Akhondzadeh S. Herbal medicines in the treatment of psychiatric and neurological disorders. In: L'Abate L, ed. *Low-Cost Approaches to Promote Physical and Mental Health*. New York, NY: Springer; 2007:119-138.
19. Akhondzadeh S, Kashani L, Fotouhi A, et al. Comparison of *Lavandula angustifolia* Mill. tincture and imipramine in the treatment of mild to moderate depression: a double-blind, randomized trial. *Progr Neuropsychopharmacol Biol Psychiatry*. 2003;27: 123-127.
20. Kooti W, Mansouri E, Ghasemiboroon M, Harizi M, Ashtary-Larky D, Afrisham R. The Effects of hydroalcoholic extract of apium graveolens leaf on the number of sexual cells and testicular structure in rat. *Jundishapur J Nat Pharm Prod*. 2014;9(4): e17532.
21. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc*. 2007;2: 322-328.
22. Grundmann O, Nakajima J, Seo S, Butterweck V. Anti-anxiety effects of *Apocynum venetum* L. in the elevated plus maze test. *J Ethnopharmacol*. 2007;110:406-411.
23. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods*. 1985;14:149-167.
24. Rezaie A, Mosavi G, Ahmadizadeh C, Jafari B. Study of sedative, preanaesthetic and anti-anxiety effects of Rosa damascene herbal extract in comparison with diazepam in rat. *Tehran Univ Med J*. 2011;69:179-184.
25. Marder M, Paladini AC. GABA(A)-receptor ligands of flavonoid structure. *Curr Top Med Chem*. 2002;2:853-867.

26. Niksokhan M, Hedarieh N, Najafifard M, Najafifard M. Effect of hydro-alcholic extract of *Pimpinella anisum* seed on anxiety in male rat. *J Gorgan Univ Med Sci*. 2014;16(4):28-33.
27. Kumar A, Agarwal P, Shakya A, Thakur AK, Kumar V. Key role of carnosic acid in the anxiolytic-like activity of *Rosmarinus officinalis* Linn. *J Pharmacol Clin Toxicol*. 2013;1(2):1013.
28. Oliván A, Munné-Bosch S. Diurnal patterns of α-tocopherol accumulation in Mediterranean plants. *J Arid Environ*. 2010;74: 1572-1576.
29. Kittur S, Wilasrusmee S, Pedersen W, et al. Neurotrophic and neuroprotective effects of milk thistle (*Silybum marianum*) on neurons in culture. *J Mol Neurosci*. 2002;18:265-269.
30. Parisa S, Abbas B, Gholmreza J. Antioxidant capacity and anti-inflammatory of alcoholic extraction of chamomile, morus, marshmallow, borage and rosemary. *J Knowledge Health*. 2013;8(1):31-34.
31. Hosseinzadeh H, Ramezani M, Shahsavand S. Effect of *Rosmarinus officinalis* L. aerial parts extract and fractions on morphine withdrawal syndrome in mice. *J Med Plants*. 2006;4(20):27-35.